CARTESIAN DIVER RESPIROMETER

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In this paper is described a modification of Linderstrøm-Lang's Cartesian diver method (1937) specifically adapted for the measurement of slight variations in the respiration of cellular objects. The diver is a further development of the diver used by Zeuthen (1946) for measuring respiratory rhythms in the egg of the frog.

Essential Features of the Method

The diver (Fig. 1) has a gas volume of only about 1 μl. It is kept floating at a constant level throughout the experiment. Therefore no disturbing currents are set up in the flotation medium by the diver itself and the saturation equilibria within the diver (see below) are not influenced by such more or less sudden changes in pressure in the gas bubble as would result if the diver was allowed to go to the bottom of the flotation vessel between the measurements. The gas volume in the diver is confined to one air bubble only. Therefore only one meniscus separates the gas space in the diver from the surrounding medium. All these factors are considered essential for obtaining accurate respiration measurements. One more factor is of paramount importance: since the diver is supposed to float in a medium which does not itself flow, it is necessary to avoid heat convection currents in the flotation vessel. Such currents will result from temperature variations in the thermostat. Not only the magnitude of possible temperature variations is important, but also the rate of variation should be kept low. A very fine thermostat, operating with as gentle heating and cooling as possible, is necessary. If not, the temperature regulation itself, usually operating on the principle of alternating heating and cooling, will result in convection currents in the flotation vessel. Therefore, in most cases it is much more convenient to work in a well-stirred water bath which is not regulated at all, but which adjusts itself to the room temperature. If the water bath is large and the room temperature fluctuates only slightly, the temperature will change very slowly. In this way no disturbing convection currents are initiated. The total change in temperature of the bath can be kept within very moderate limits if the room temperature is relatively constant. The change in pressure in the diver is automatically adjusted for temperature changes because the other side of the manometer is connected to the enclosed gas volume in a large bottle (2 liters).

The diver consists of a diver chamber, bottom droplet with animals, gas bubble, and alkaline, CO_2-absorbing neck fluid. The gas bubble forms a complete separation between bottom and neck fluids (this point is discussed at some length by Giese and Zeuthen (1949)). A hollow glass stopper is inserted in the neck of the diver. The stopper serves several different purposes: it retards diffusion of gases to or

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from the surroundings, gives the diver buoyancy and permits the pressure-sensitive air volume of the diver to be reduced almost ad libitum. Since the glass stopper is inserted without any grease, narrow passages are left which allow the air bubble in the diver to react with volume changes to outside pressure variations, created by the aid of an adjustable water manometer. The diver floats in the same medium which is in the neck of the diver; e.g., in alkaline water, isotonic with the biological medium in the bottom droplet. A long glass extension may be attached to the outer end of the stopper. This makes the diver float upside down ("inverted diver"), so that the organisms in the diver, if immobile, will come to rest on the meniscus between air and water, not on the bottom of the diver, away from the air bubble.

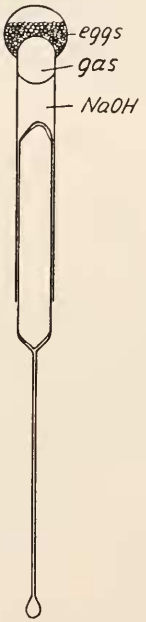

FIGURE 1. The diver.

Experiments with the diver are reported in the paper by Zeuthen (1950b). Figure 2 in that paper indicates that the diver is practically tight to gases. However, in the first part of the experiment, an apparent respiration is often measured. This is explained by assuming that a diffusion equilibrium is gradually becoming established inside the diver. If, for instance, the diver is filled with fluids which are in equilibrium with air, but an oxygen bubble is introduced, gas goes in solution because oxygen is more soluble in water than is air: Equilibrium is established asymptotically. How long it takes before it is well enough established depends mainly on the gases involved, on the relative amounts of water and gas in the system, and on the diffusion distances in the water of the diver. In the diver used for the experiments on eggs of *Urechis,* relatively much water was present in the system. Therefore the initial equilibration period was long (1–2 hours). It is important to remember that for disclosing a possible rhythm in respiration, the initial period need not be over. If it is not over, the rhythmic respiration is merely superimposed on a smooth curve which asymptotically approaches the X-axis.

During the respiration experiment, the pressure in the system decreases. At the same time as oxygen is used from the bubble, gas which is dissolved in the water in the diver again goes out of solution, back to the bubble. The condition of equilibrium spoken of becomes a quasi-equilibrium in which the oxygen tension in the water and in the bubble drops at the same rate. Thus oxygen is taken from water and from bubble in proportion to the amounts of oxygen present in both places. In the author's experiments on *Urechis,* approximately 6 to 10 per cent of the oxygen was present in the water, and the total respiration is measured too low by this value. For the measurement of variations in oxygen uptake this means practically nothing (comp. Linderstrøm-Lang, 1946). However, as soon as absolute respiration measurements are to be performed, these conditions have to be taken into consideration by applying corrections or by reducing the ratio between water and gas in the diver.

Any variation in respiratory rate must show up somewhat delayed because a diffusion path is introduced between the respiring objects and the air bubble. In the inverted diver (with objects on the meniscus) this is usually not a significant factor, however. In the experiment on *Urechis* (diffusion distance less than 0.5 mm) the delay was probably less than 1–2 minutes. Moreover, the fluctuations in respiration were recorded without any significant damping. These statements are theoretical deductions based on Linderstrøm-Lang's calculations (1946). However, the experiments of Blinks and Odenheimer (personal information), who used this diver in studies on photosynthesis in single algal cells, seem to verify these deductions. If the dimensions of the diver are changed from those shown in Figure 1, Linderstrøm-Lang's paper (1946) on diffusion systems in micro respirometers should be consulted.

Making, Calibrating and Filling the Diver

The diver is made from a thin-walled capillary tube with a diameter conveniently a little less than 1 mm. The ratio between inner and outer diameter should be close to 0.9. Such capillaries may be drawn from thin-walled test tubes which should be heated by rotation in a broad flame. From the thin-walled capillary two pieces are selected, of which one fits tightly into the other. The former piece is used for the stopper, the latter for the diver chamber. Principles of glass blowing which are given by Holter (1943) are employed. For the micro-burner a hypodermic needle is very convenient. With rich natural gas (as in California), the oblique tip of the needle serves to mix the gas and air. It is best to work with a very small flame (3–4 mm long) and it is often useful to mount the micro burner on the table of a dissection microscope. It is desirable to keep the volume of the sealed stopper small (3–4 times the volume of the "pressure sensitive" gas bubble) in order to make the diver as sensitive to pressure changes as possible.

Before use the diver should be tested and calibrated. For this purpose the diver chamber is filled completely with water and the stopper is inserted below water. Since the cross section of the capillary used for making the diver is usually not quite circular, the stopper will only slip into the diver chamber if it is correctly oriented. It requires some practice to find this orientation, and it may be useful to mark both tubes conveniently. This, however, has not been done by the author because very often useful reference marks (small dots, easily recognizable curvatures and the like) can be found on the diver. The diver thus filled should sink in its normal flotation

medium. If so, small air bubbles are introduced into the diver chamber until the diver floats. For the introducing of air bubbles the diver is taken out of the medium and the stopper is gently pulled back, without completely removing it, however. Small air bubbles will then enter the diver along the stopper. If the gas volume of the floating diver is too large or too small, glass is removed from or added to the tail of the stopper. The many small air bubbles in the diver are allowed to unite into one. Then, below water, the stopper is pulled out and the air bubble is taken into a calibrated Holter braking pipette (Holter, 1943). The amount of air to be introduced into this diver before each experiment is indicated by the length of the air column in the braking pipette used.

A great advantage of the present method is that the diver can be charged free-hand. Before the experiment the diver is rinsed by sucking first sulfuric acid and then water through the diver chamber by means of an ordinary pipette which is introduced into the submerged diver chamber. The measured volume of air is taken up into the braking pipette, and after this the biological object(s). In the filled pipette, therefore, we have in the lower end the respiring cells, and in the upper end the measured air volume. The pipette is gently blown out into the diver chamber which has previously been completely filled with water. If done under the dissection microscope it is fairly easy to deposit a large number of cells in the bulb of the diver and to place the air bubble where desired in the capillary, thus trapping the cells. After the experiment the cells may be removed from the diver and counted. It has (rather unexpectedly) been found that a complete and safe separation between bottom droplet and neck seal is formed if the air bubble is big enough to make a circular but very short (about 0.2–0.3 mm.) zone of adherence to the glass wall. Creeping of fluid along the glass from neck to bottom has only been observed when dust particles happened to form visible bridges across the clean glass surface. After the neck fluid has been replaced with isotonic alkali which is run in with a pipette (care being taken not to touch the air bubble), the stopper is introduced and the diver is transferred by pipette into the flotation vessel. The flotation vessel, manometer, microscope, and thermostat, have been described by Holter (1943); for thermostat, however, see above.

Measuring the Equilibrium Pressure

When the respiration of an object is to be measured over short periods the equilibrium pressure as well as the time must be measured very accurately. The following procedure of "turning point determination" has given good results: the diver floats at about the same level all the time. Just before a reading is to be taken, the manometer is adjusted at such a constant level that the diver rises very slowly. However, due to the respiration processes, the diver becomes gradually heavier, so that it rises more and more slowly, comes to a standstill, and finally starts sinking. It is possible to make the diver turn at exactly the same level ($\pm$ a few 0.01 mm.) in all measurements. When the diver turns, a stopwatch is started. The watch is stopped again when the next reading is due, and at the same time another stopwatch is started for measuring the next period, etc. In this way the pressure remains unchanged from just before to just after the diver turns, and the two branches of the manometer can therefore be read (to the nearest 0.1 mm.) immediately after the watches have been pressed. The equilibrium pressure measured is valid for the

moment when the diver turns and this moment can be observed with an accuracy of some few seconds. The described method of measuring equilibrium pressures works best when the equilibrium pressure changes fast.

Sensitivity of Method

The accuracy of the method was studied in connection with the experiments on dividing *Urechis* eggs. With a diver having an air volume of 1 μl. the oxygen consumption measured in 5-minute periods was about $2.5–5 \times 10^{-3}$ μl. (change in equilibrium pressure 2.5–5 cm./5 min.). This respiration could be measured with an error of probably well below 1 per cent. Thus the sensitivity of the method is about $2.5–5 \cdot 10^{-5}$ μl.

Summary

A Cartesian diver method which is specially adapted for following variations in respiratory rate of small objects is described. Accurate respiration measurements can be obtained every few minutes. The diver is calibrated empirically and it is filled free-hand.

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